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Characterization of Potential Pathogenic Bacterial Isolates from Urban and Rural Market Dumpsites

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Abstract: Antimicrobial susceptibility and toxigenicity tests were carried out on bacterial species isolated from the soil samples collected from an urban market (Oba market) and a rural market (Ekiadolor market) waste dumpsites. The bacterial species included *Escherichia coli, Shigella sp, Staphylococcus aureus, Salmonella sp, Bacillus sp, Enterococcus sp, Clostridium sp, Proteus sp, Klebsiella sp* and *Pseudomonas sp. E. coli* had the highest percentage occurrence of 56.0% in the urban market waste dumpsite and *Enterococcus sp* had the highest percentage occurrence of 28.13% in the rural market waste dumpsite. Toxigenicity test was carried out on *S. aureus, Salmonella sp* and *E. coli* isolates to ascertain enterotoxin production using the rabbit ileal loop assay. *S. aureus* (60%), *Salmonella sp* (50%) and *E. coli* (62.5%) isolates were positive to enterotoxin production. Ciprofloxacin (CIP), Peflacin (PEF), Tetracycline (TET) and Gentamycin (GEN) were the most effective antibiotics against the bacterial isolates. Antimicrobial susceptibility of the bacterial isolates from the urban waste dumpsite was CIP (40.74%), PEF (35.19%) and GEN (33.33%) and antimicrobial susceptibility of the bacterial isolates from the rural waste dumpsite was CIP (51.43%), TET (40.00%) and GEN (42.86%). The antibiotics were more effective against the bacterial isolates from the rural market dump site. Pollution of the environment by wastes from markets is of public health concern because the presence of pathogenic bacteria in the wastes has the potential of contaminating air, soil and ground water.

Keywords: Bacterial Isolates, Dumpsites, Market, Pathogenic, Rural, Urban

1. Introduction

Refuse dumps refer to areas or land sites where wastes from several sources and processed are deposited [1]. Markets in Nigeria are characterized by open dumpsites with wastes often allowed to pile up until they are taken by waste management officials after some time. Refuse dumps provide a rich source of microorganisms most of which are pathogenic [2]. These microorganisms in the waste dumps use the waste constituents as nutrients [3]. The refuse dumps therefore attract rodents and vector insects capable of transmitting pathogenic agents of diseases such as typhoid fever, diarrhea, cholera, dysentery, salmonellosis in humans and animals. Infections are caused by bacteria which are suspended in air around these refuse dumps [1] especially during buying or selling near these waste dump sites. Also, the unsightly accumulation of these wastes reduces the aesthetic value of the markets. The soils underneath become poor in organic matter and fertility with reductions in their most important physical properties such as structural stability and water reaction [4]. The presence of pathogenic bacteria and maybe harmful chemicals in the market waste dumpsites is of public health significance. The risk of acquiring a disease when buying or selling in a market where waste dumpsite exist is difficult to estimate but mortalities can occur with these bacterial organisms in the form of secondary infections [5].

Antibiotic resistant bacteria are extremely important to human health and have become a major public health challenge globally [6, 7]. Studies by [6] showed that high

prevalence of bacteria resistant to most commonly used antibiotic were present in dumpsites. They ascertained that the dumpsite was composed of solid wastes from different sources and therefore expected that microbes found therein were brought to the dumpsites along with the wastes. The objective of this study is to determine the potential pathogenic bacteria isolates from urban and rural market dumpsites and determine their level of susceptibility to antimicrobial drugs.

2. Materials and Methods

2.1. Collection of Soil Samples

Soil samples were collected from the waste dumpsites of an urban market (Oba market) and a rural market (Ekiadolor market) in Benin City (Long 5'40° E and Lat 6'00° N), Edo State, Nigeria. The samples were collected from the soil inside the waste dumps at depth 0 – 10 cm using a hand trowel cleaned with 70% alcohol into polyethylene bags. The samples were treated within 2 h of collection [3]. Where immediate bacteriological examinations were not possible, samples were stored in a refrigerator at 4°C until they were examined in the laboratory [8].

2.2. Bacteriological Analyses

Cultivation and enumeration of the total viable heterotrophic bacteria count was done in duplicates. One gram (1 g) of the soil sample was weighed and mixed thoroughly in 10 ml distilled water. Then 1 ml of the dilution was transferred aseptically into another test tube containing 9 ml distilled water and diluted serially into other test tubes till a 10^{-5} dilution was made. An aliquot of 0.1 ml of each dilution was aseptically plated out using pour plate method on Tryptone Soy Blood Agar (Lab M Ltd UK), Desoxycholate Citrate Agar (Lab M Ltd UK) and Nutrient Agar (Lab M Ltd UK). All plates were incubated at 37°C for 24°C to 48°C to obtain total bacterial counts. Confirmation of coliform organisms was carried out by inoculating colonies into lactose broth with Durham tubes at 37°C for 48 h to evaluate gas formation [9]. The lactose broth in the tubes with gas was streaked out on Eosin Methylene Blue Agar (Lab M Ltd UK) at 37°C for 24 h. The discrete colonies on the different agar media were observed and counted in a Techmel and Techmel counter model TT 201. The results were expressed as colony forming units per gram (cfu/g). Different bacterial colonies were subcultured on Nutrient Agar plates at 28°C for 24 h. Pure colonies were transferred aseptically on Nutrient Agar slants and incubated at 28°C for 24 h. Representatives of the different purified colonies were subjected to various cultural, morphological and biochemical tests. Identification was based on Bergey's Manual of Determinative Bacteriology [10].

2.3. Preparation of Enterotoxin Supernatant

Preparation of enterotoxin from *Salmonella sp, S. aureus* and *E. coli* was determined using the method of [11]. The bacteria isolates were inoculated each into 5 ml of nutrient broth in 15 x 125 mm sterile screw cap test tubes. The cultures were incubated at 37°C for 24 h. Then 0.1 ml of each test organism was inoculated into 10 ml of milk at pH 8 and pasteurized by heating to 80°C for 30 min [11]. The cultures were then incubated at 37°C for 48 h. The cell free supernatants were collected by centrifugation at 5000 rpm for 5 min and then decanted into sterile test tubes. The cell free supernatant of the test organisms were then used as crude toxin preparations [12].

2.4. Enterotoxin Production Using Rabbit Ileal Loop Assay

The method of [13] was used to assay *Salmonella sp, S. aureus* and *E. coli* for enterotoxin production. Three healthy white adult rabbits weighing less than 2 kg were anaesthetized. The peritoneal cavity of each rabbit was opened aseptically by surgical technique and the bowel was carefully washed with pre - warmed sterile water. The length of the bowel making the loops was cut at both ends and all cut surfaces were clamped. The ileal loops were ligated into 5 cm segments with 2 cm intervals. The ends of the isolated intestine were closed with sutures and the required number of loops was constructed with ligatures. Then 0.5 ml of the crude toxin supernatant of the test organisms was injected into each of the ligated segments of the ileum. Some segments were injected with 0.5 ml of sterile water to serve as control segments. The loops were replaced in the peritoneal cavity in their original positions and the peritoneum closed. The animals were again anaesthetized 18 h after inoculation and the peritoneum cavity opened again [13]. Positive loops showed intense inflammatory response and fluid accumulation in segments that contained enterotoxigenic organisms. The volume of the fluid recovered using syringes was used to determine the dilatation index (DI), which is estimated as the ratio of the volume of the fluid accumulated in the intestinal loop to the length of the ileal segment of the loop. A DI greater than 0.2 ml/cm was then reported as positive [11].

2.5. Antimicrobial Susceptibility Test

The disc diffusion susceptibility method [14] was used to study the antimicrobial susceptibility of the bacterial isolates from the urban and rural markets waste dumpsites. The commercially prepared antibiotics used included Amoxycillin (10 µg), Cotrimoxazole (30 µg), Peflacin (30 µg), Oflaxacin (5 µg), Augmentin (30 µg), Nirofurantoin (300 µg), Tetracycline (30 µg), Gentamicin (10 µg), Erythromycin (30 µg), Cloxacillin (5 µg), Ampicillin (10 µg), Streptomycin (30 µg), Ceftazidime (30 µg) and Ciprofloxacin (5 µg). Overnight broth cultures of the pure bacterial isolates were suspended into sterile peptone water. Using a sterile swab stick, the inoculums were inoculated into the surface of dried plates of Mueller – Hinton agar and rotated carefully to ensure even distribution [15]. The surface of the each plate was allowed to dry for few minutes and with sterile forceps, the discs were placed on the inoculated plates.

The plates were then incubated at 37°C for 24 h. The diameter of the zone of inhibition of the discs were measured to the nearest whole number and interpreted on the basis of CLSI guideline [16].

3. Results and Discussion

The mean population (cfu/g) and the percentage occurrence of the bacterial isolates from Oba market (urban market) and Ekiadolor market (rural market) dumpsite are presented in Tables 1 and 2. All the bacterial species in the urban market waste dumpsite had higher counts and percentage occurrence than in the rural market waste dumpsite except *Shigella sp* and *Proteus sp.* This could be as a result of higher waste generation from the increased population and residential areas in the urban areas. The level of pollution from the heap of refuse dumps is higher in the urban market as some of the residential buildings around the markets have no toilets or waste disposal facilities, so they use the market as dumping ground [17]. The potentially pathogenic bacteria agents isolated in this study is in agreement with the studies of [3, 17] and human diseases originated from these community acquired bacteria agents where environmental conditions such as poor sanitation, heavy flies' density and indiscriminate disposal of human and animal wastes are prevalent [18]. The heap of refuse dumpsites in the rural and urban markets are located in the market centre where arrays of exposed food items are displayed and the reservoir of pathogenic infectious agents in the dumps portend great danger to public health [19]. The high prevalence of *E. coli* and *Enterococcus sp* in the urban and rural market dumpsites indicated that human and animal faecal wastes were dispersed with the refuse. This is capable of causing outbreak of food and water borne diseases [19]. The highest percentage frequency of occurrence of *E. coli* in the urban market waste dumpsite was in agreement with the reports of [18] which stated that *E. coli* is able to withstand competition from the indigenous microorganisms with higher growth rates. Also, the uncontrolled manner in which solid wastes were disposed off at most open dumpsites created serious environmental degradation translating into economic, social and health issues [4]. Therefore, the refuse wastes could be properly harnessed by bioconversion into organic fertilizer for use in gardening, agriculture and horticulture [3].

Table 1. *Mean Bacterial Counts (cfu/g) from Soil Samples of Oba market and Ekiadolor Market Dumpsites.*

Bacteria Isolates	Oba Market	Ekiadolor Market
E. coli	$16.39 \times 10^4 \pm 0.45$	$44.77 \times 10^3 \pm 0.11$
Shigella sp	$18.26 \times 10^3 \pm 0.30$	$25.58 \times 10^3 \pm 0.35$
S. aureus	$73.08 \times 10^3 \pm 1.10$	$31.99 \times 10^3 \pm 0.80$
Salmonella sp	$54.81 \times 10^3 \pm 1.05$	$25.59 \times 10^3 \pm 1.03$
Bacillus sp	$45.69 \times 10^3 \pm 0.50$	$19.19 \times 10^3 \pm 1.10$
Enterococcus sp	$13.71 \times 10^4 \pm 0.05$	$57.55 \times 10^3 \pm 1.05$
Clostridium sp	$27.42 \times 10^3 \pm 0.84$	$12.79 \times 10^3 \pm 0.01$
Proteus sp	$18.29 \times 10^3 \pm 0.01$	$31.98 \times 10^3 \pm 1.50$
Klebsiella sp	$18.29 \times 10_3 \pm 0.01$	0.00 ± 0.00
Pseudomonas sp	$82.22 \times 10^3 \pm 1.00$	$38.36 \times 10^3 \pm 1.00$

Table 2. *Mean Percentage Occurrence of Bacterial Isolates from Soil Samples of Oba Market and Ekiadolor Market Dumpsites.*

Bacteria Isolates	Mean Percentage Occurrence (%)	
	Oba Market	Ekiadolor Market
E. coli	56.0	21.88
Shigella sp	6.25	12.50
S. aureus	25.00	15.64
Salmonella sp	18.75	12.51
Bacillus sp	15.63	9.38
Enterococcus sp	46.89	28.13
Clostridium sp	9.38	6.25
Proteus sp	6.26	15.63
Klebsiella sp	6.26	0.00
Pseudomonas sp	28.13	18.75

The isolation of *Bacillus, Staphylococcus, Klebsiella* and *Proteus* species from the market dumpsites is in agreement with the studies of [20]. These microorganisms produce enzymes like Dnase and Staphylokinase that could degrade wastes at dumpsites and convert them to useful materials [21].

In Table 3 is presented the enterotoxin assay on *E. coli, S. aureus* and *Salmonella* species using rabbit ileal loop method (RILM). The production of enterotoxins by these pathogenic bacteria species cause serious health problems when ingested and inhaled by humans. Staphylococcal enterotoxins are responsible for food poisoning, acute illness, fever, erythematous lesions and hypertension [22]. Strains of *Salmonella* cause illnesses such as typhoid fever and salmonellosis due to ingestion of contaminated food [23]. *E. coli* toxins are responsible for numerous reports of contamination of foods and beverages causing diarrhea, urinary tract infections, respiratory illnesses and gastrointestinal infections [24]. The RILM has been used to study the histopathological changes associated with human enterotoxicosis and various diarrhoeal diseases [13, 25]. This study showed that 62.5%, 60% and 50% of the *E. coli, S. aureus* and *Salmonella* crude enterotoxins respectively elicited positive response in ileal loops of the rabbits with DI values that ranged from 0.29 to 0.42 ml/cm. This is in agreement with the studies of [11] who reported a DI of 0.2 to 0.48 ml/cm when enterotoxigenic *S. aureus* was introduced into rabbit ileal loops. The colour and viscosity of the accumulated fluids in the ileal loops after 24 h were pinkish (blood stained), yellowish, brownish and very watery when compared to the control loops that were white and viscous. The induced accumulated fluids showed destruction of the villus structure with haemorrhage in the layer of the mucosa [26], observations consistent with the inflammatory type of illness seen in man [11]. The release of enterotoxins from these organisms isolated from the rural and urban market waste dumpsites (where buying and selling take place with the foods mostly exposed) are capable of causing outbreak of food and water borne diseases through the contaminative route [27].

The antimicrobial susceptibility patterns of the bacterial isolates in Oba market and Ekiadolor markets are presented in Tables 4 and 5. Findings from this study showed that ciprofloxacin, peflacin, tetracycline and gentamycin were the

most effective antibiotics against the bacteria. The bacterial isolates from the urban market dumpsite showed 40.74%, 35.19% and 33.33% susceptibility to ciprofloxacin, peflacin and gentamycin and the bacterial isolates from the rural market dumpsite showed 51.43%, 40.00% and 42.86% susceptibility to ciprofloxacin, tetracycline and gentamycin. Ciprofloxacin was the most effective antibiotic to *Salmonella sp, S. aureus, Klebsiella sp* and *Proteus sp.* This is in agreement with the study of [8]. The bacterial isolates were highly resistant to the antibiotics especially streptomycin, erythromycin, augmentin and ampicillin. The bacterial isolates may have been resistant because of the production of enzymes which inactivate or modify antibiotics, cause changes in bacteria cell membrane, modification of target site and development of metabolic pathways by bacteria [28].

Table 3. *Enterotoxin Assay of Test Organisms from Urban and Rural Market Dumpsites Using Rabbit Ileal Loop Method.*

Test Organisms	Isolates	Content of ileal loop	Measurement of fluid (ml)	Dilation Index DI (ml/cm	Enterotoxin response
E. coli	1	Slightly viscous yellow to very brownish and watery fluid	2.12	0.42	+
	2		0.85	0.17	-
	3		0.63	0.13	-
	4		2.06	0.41	+
	5		1.46	0.29	+
	6		1.75	0.35	+
	7		0.94	0.19	-
	8		1.96	0.39	+
S. aureus	1	Milky yellow to very brownish watery fluid	2.01	0.40	+
	2		0.97	0.19	-
	3		0.92	0.18	-
	4		2.00	0.40	+
	5		1.70	0.34	+
Salmonella sp	1	Slightly viscous to pinkish watery fluid	1.65	0.33	+
	2		1.68	0.34	+
	3		0.58	0.12	-
	4		0.69	0.14	-
Control	0.5 ml sterile water	White and viscous fluid			

The origin of this widespread resistance by all the isolated may have resulted from natural production of antibiotics by soil organisms, run off from animal feed, crops or waste products from treated livestock or humans [29]. The prevalence of multidrug resistance by the bacterial isolates from the dumpsites suggests that there is a high chance of spreading these pathogens and the associated resistant genes to humans and animals [6]. The high population of people in market places daily and those close to the dumpsites present a high risk of spreading the antibiotic resistant genes in the pathogens to humans. The infections caused by such bacteria are very difficult to treat [29].

Table 4. *Antimicrobial Susceptibility Pattern of Bacterial Isolates in Oba Market Waste Dumpsite.*

Bacteria isolates	Number of isolates	Percentage of isolates Susceptible to Antimicrobial Drugs (%)													
		AMX	COT	PEF	OFL	AUG	NIT	TET	GEN	ERY	CXC	AMP	STR	CAZ	CIP
E. coli	8	25.00	25.00	37.50	-	0.00	50.00	25.00	50.00	-	0.00	25.00	-	-	-
Shigella sp	4	50.00	50.00	-	0.00	0.00	0.00	75.00	25.00	-	-	0.00	-	50.00	50.00
S. aureus	5	-	40.00	60.00	-	0.00	0.00	-	0.00	40.00	20.00	0.00	-	60.00	80.00
Salmonella sp	4	100.00	-	50.00	75.00	-	-	50.00	-	-	0.00	0.00	-	-	100.00
Bacillus sp	7	0.00	0.00	42.86	-	0.00	-	0.00	42.86	0.00	0.00	0.00	14.29	-	28.57
Enterococcus sp	6	0.00	0.00	33.33	-	0.00	50.00	33.33	-	50.00	-	16.67	33.33	-	33.33
Clostridium sp	5	0.00	0.00	0.00	60.00	20.00	40.00	-	-	20.00	20.00	-	-	0.00	-
Proteus sp	5	-	0.00	-	-	40.00	0.00	60.00	60.00	-	20.00	-	-	0.00	60.00
Klebsiella sp	4	-	25.00	50.00	-	-	25.00	50.00	75.00	-	-	-	-	75.00	100.00
Pseudomonas sp	6	0.00	-	66.67	33.33	33.33	0.00	0.00	66.67	-	-	-	0.00	-	0.00
Total	54	14.81	12.96	35.19	14.81	9.26	18.52	27.78	33.33	11.11	5.57	5.57	7.41	14.81	40.74

Key: - = organism yielded no growth 0.00 = not susceptible AMX = Amoxycillin COT = Cotrimoxazole PEF = Peflacin OFL = Ofloxacin AUG = Augmentin NIT = Nitrofurantoin TET = Tetracycline GEN = Gentamycin ERY = Erythromycin CXC = Cloxacillin AMP = Ampicillin STR = Streptomycin CAZ = Ceftazidime CIP = Ciprofloxacin

Table 5. *Antimicrobial Susceptibility Pattern of Bacterial Isolates in Ekiadolor Market Waste Dumpsite.*

Bacteria isolates	Number of isolates	Percentage of isolates Susceptible to Antimicrobial Drugs (%)													
		AMX	COT	PEF	OFL	AUG	NIT	TET	GEN	ERY	CXC	AMP	STR	CAZ	CIP
E. coli	6	66.67	66.67	50.00	-	-	-	66.67	66.67	-	16.67	-	0.00	50.00	66.67
Shigella sp	3	33.33	33.33	-	-	0.00	-	66.67	66.67	-	-	0.00	-	66.67	66.67
S. aureus	4	25.00	50.00	75.00	-	-	-	50.00	0.00	-	25.00	0.00	0.00	0.00	50.00
Salmonella sp	3	66.67	0.00	-	66.67	0.00	-	33.33	33.33	-	-	33.33	-	-	66.67
Bacillus sp	4	-	-	75.00	50.00	-	-	25.00	-	0.00	25.00	0.00	0.00	0.00	-
Enterococcus sp	4	25.00	-	75.00	-	-	-	25.00	-	25.00	0.00	25.00	50.00	-	75.00
Clostridium sp	2	0.00	0.00	50.00	100.00	50.00	100.00	50.00	0.00	-	-	-	0.00	0.00	-
Proteus sp	5	-	20.00	0.00	-	-	-	20.00	100.00	-	60.00	0.00	-	100.00	100.00
Pseudomonas sp	4	-	0.00	-	-	0.00	-	0.00	75.00	-	50.00	-	0.00	50.00	0.00
Total	35	25.71	22.86	37.14	17.14	2.86	5.71	40.00	42.86	2.86	22.86	5.71	5.71	34.29	51.43

Key: - = organism yielded no growth 0.00 = not susceptible AMX = Amoxycillin COT = Cotrimoxazole PEF = Peflacin OFL = Ofloxacin AUG = Augmentin NIT = Nitrofurantoin TET = Tetracycline GEN = Gentamycin ERY = Erythromycin CXC = Cloxacillin AMP = Ampicillin STR = Streptomycin CAZ = Ceftazidime CIP = Ciprofloxacin

4. Conclusion

The urban and rural market dumpsites were unsightly and emitted foul odour. The wastes contained pathogenic bacteria that were resistant to antimicrobial drugs. There is possible risk of the spread of these pathogens to humans causing varying food and water borne diseases and respiratory diseases. Market wastes should therefore be properly be managed and disposed to avoid the environmental and health impact of such wastes. There should be health education about the importance of daily clean up and removal of market wastes.

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